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A SUMMARY OF INVERTEBRATE LEUCOCYTE MORPHOLOGY WITH EMPHASIS ON BLOOD ELEMENTS OF THE MANILA CLAM, *TAPES SEMIDECUSSATA* [1]

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Metchnikoff (1968, page 76) in 1891 defined leucocytes as ". . . colorless cells, possessing one or rarely two nuclei and a protoplasmic body capable of amoeboid movement. In many invertebrates there is only one variety of mobile blood corpuscles containing a few sparse granules, whereas in certain others, such as many insects and molluscs, two varieties occur—granular leucocytes, with a large number of coarse granules, and hyaline leucocytes, with few or no granules."

Within the broad range of this definition, the literature has been abundantly supplied with an array of names assigned to leucocytic cells. Up to 76 different morphological varieties of cell types were classified from lamellibranch molluscs in one paper (Betances, 1922). A few general reviews are available for information in invertebrate hematology. Of some use are Andrew (1965), Hyman (1967) and other volumes in the same series; Grassé (1960) and other volumes in this series; Maupin (1969); and Cuénot (1891, 1897).

In this study, the morphology and morphometrics of the leucocytes of *Tapes semidecussata* are described and related to criteria for general leucocyte forms. The literature describing leucocyte form and function within the invertebrate phyla is summarized in Table I. This table is not a complete review of the known literature but is an attempt to reduce the number of named forms to broad categories of similar cytology and function.

Methods and Materials

The Manila clam (Japanese littleneck, Philippine clam), *Tapes semidecussata* (*Protothaca* (*Venerupis*) *japonica* Deshayes; *Paphia philippinarus* Adams and

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TABLE I

Invertebrate leucocytes from Porifera to Chordata

Phylum and Class	General cell type	Published names and synonyms	Where observed	Morphology	Size	Properties and special characteristics
Porifera (1, 35)	Hyaline leucocyte	Scleroblast (1)	Mesoglea			Secrete spicules
		Archeocyte (35)	Mesoglea	Large nuclei, slight cytoplasm		Stem cell, phagocytosis and encapsulation
		Amoebocyte (1)	Mesoglea	Variable, cytoplasm with or without granules		Phagocytosis, nutritive and defense
Coelenterata (1)	Hyaline leucocyte	Interstitial cell (1)	Mesoglea	= Archeocyte		Stem cell, give rise to cnidoblasts, *etc.* Originate from endo- and exodermal cells.
Platyhelminthes (1, 36)	Leucocyte	Neoblast	Mesoderm			Stem cell or intermediate dedifferentiated cell with totipotency
Aschelminthes Rotifera (1)	Hyaline leucocyte	Ameboid cells (1)	Pseudocoel	Branched pseudopodia		Phagocytic, excretory?
Nemertea (1)	Hyaline leucocyte	Lymphocyte	Mesenchyme	Similar to those in circulatory system		Regeneration, gonad formation
		Hyaline amoebocyte	Circ. system			
	Granulocyte	Granular amoebocyte	Circ. system			
	Erythrocyte	"erythrocyte"	Circ. system	Flattened, round or oval		Oxygen transport
Mollusca Polyplacophora (1, 2, 3)	Hyaline leucocyte	Leucocyte (3)	Hemolymph	Round or oval cells, weakly basophilic cytoplasm	4–8 μ	Seasonal variations in formed elements noted
	Leucoblast	Leucoblast	Hemolymph	Smaller with strongly basophilic cytoplasm		
Monoplacophora (3)	Granulocyte	(3)	Hemolymph	Round or slightly oval cells with a few slightly basophilic granules in the cytoplasm	7–10 μ	*Neoplina galatheae*
Lamellibranchia (1, 2, 4, 5, 6, 7, 8, 9)	Hyaline leucocyte	Macronucleocyte	Hemolymph (smears)	Hyaline cytoplasm = vert. lymphocyte. Nuc. $\frac{1}{2}$ to $\frac{2}{3}$ dia. of cell. Chromatin clumped	10–20 μ dia.	7.5% of total FW mussels
	Acidophil	Granular eosinophilic amoebocyte	Hemolymph (smears)	Nuc. $\frac{1}{3}$ size of cell, eccentric & ovoid; nucleolus often visible. Chromatin lightly clumped	8–20 μ dia.	85% of total FW mussels
	Basophil	Granular basophilic amoebocyte (7)	Hemolymph (smears)	Nuc. $\frac{1}{3}$ to $\frac{1}{2}$ size of cell. Chromatin heavily clumped	7–12 μ dia.	7% of total FW mussels
	Granulocyte	Granulocyte (4)	Hemolymph	= Crustacean forms		*Cardium, Pecten maximus*
	Leucocyte	Lymphocyte (4)	Hemolymph	= Mammalian forms		*Cardium, Pecten maximus*
	Granulocyte	Coarse grained amoebocyte (5)	Hemolymph (smears & sections)	Birefrigent granules, colored or colorless, depending on species	8 $\times$ 12 μ	Precursor to two below?
	Granulocyte	Fine grained amoebocyte (5)	Hemolymph (smears & sections	Birefrigent granules, colored or colorless, depending on species	8 $\times$ 12 μ	

TABLE 1—(*Continued*)

Phylum and Class	General cell type	Published names and synonyms	Where observed	Morphology	Size	Properties and special characteristics
	Leucoblast?	Basophilic (5) amoebocyte (5)	Hemolymph (smears & sections	Narrow band of cytoplasm, no granules	8×12 μ	
	Erythrocyte	"erythrocyte"	Hemolymph	Variable shape	20×30 μ	Oxygen transport? Numerous in *Arca*.
	Acidophil	Coarsely granular amoebocyte (6)	Hemolymph (smears)	Large amt. cyto. granular eosinophila not in cytoplasm. Round to oval central nucleus		Largest form. *Cardium norvegicum*. 48% of tot.
	Acidophil	Finely granular eosinophil (6)	Hemolymph (smears)	Coarse eosinophilic granules, well defined		Slightly smaller. 44% of total
	Basophil	Basophilic corpuscle (6)	Hemolymph	Round nucleus and small amount of cytoplasm, basophilic		Smallest. 8% of total
	Granulocyte "Brown cell"	Brown cell (8)	Auricular pericardial gland	Applique nuc. (2.6×4.4 μ). Small amt. of clear cyto. Large vacuole (s) with brown pigment. No nucleolus	11×18 μ	Cell prob. related to or arise from leucocytes. Function in excretion, protection, defense?
	Granulocyte	Granular amoebocyte (9)	Hemolymph	Granules, yellow or yellowish-green. Ovoid nuc. Neutro-baso-philic cytoplasm fixed	9–13 nuc. 3×4 μ	Modified amoebocyte. Amoeboid but slow
	Leucocyte	Hyaline amoebocyte (9)	Hemolymph	= to above when fixed and stained. Ovoid nucleus	5–15 μ nuc. 5 μ	Function in phagocytosis and pinocytosis
Gastropoda (1, 2, 10, 11, 12, 14)	Leucocyte	Hyaline amoebocyte (2)	Hemolymph (smears)	Small, might enlarge to one filled with eosinophilous granules		*Viviparus*
	Leucocyte	Lymphoid cells (10)	Hemolymph) (smears)	Usu. round, sometimes spindle-shaped. Thin margin of hyaline cytoplasm	3–7 μ	Living cells exhibit amoeboid activity with filiform and lobose pseudopodia. *Busycon*
	Granulocyte	Granular macrophage (10)	Hemolymph (smears)	Many neutrophilic granules. Nuc. w/o nucleoli, oval, bean-shaped or bilobed	10–12 μ	Most numerous, Moderately thigmotatic and filamentous, petaloid and lobose pseudopodia
	Acidophil	Eosinophilic granular amoebocyte	Hemolymph (smears)	Cytoplasm filled with well-defined granules. Nuc. usu. eccentric, round to oval	10 μ	Cell fragments were often noted as vesicles. 1 to 2% of total
	Leucocyte	"normal cells" (11)	Hemolymph (sections and smears)	Round cells with one or more "flat" pseudopodia	10–15 μ	*Lymnaea stagnalis*
		Leucocyte (11)	Hemolymph (sections and smears)	Are a phase of normal cells		*Lymnaea stagnalis*
		"small cells" (11)	Hemolymph (sections and smears)	W/o pseudopodia. May be an artifact and arise by breakup of normal cells		*Lymnaea stagnalis*

TABLE I—(*Continued*)

Phylum and Class	General cell type	Published names and synonyms	Where observed	Morphology	Size	Properties and special characteristics
	Granulocyte	Wandering cells (11)	Hemolymph	Large and granulated-possibly phagocytic "normal cells"		*Lymnaea stagnalis*
		Small rounded amoebocytes (11)	Hemolymph	Motile and contain granules		*Lymnaea stagnalis*
	Leucocyte and granulocyte	Amoebocyte (12)	In tissue	Considerable variation in size and form. Nuc. vesicular, round, oval or lentiform. Cyto. lightly basophilic with granules. Nuc. usu. eccentric with coarse chromatin	9×12 μ	No differential count. Very little activity was correlated with digestion and shell repair. Main activity related to defense
	Small leucocyte	Small amoebocyte Type A (14)	All tissues	Hyaline cytoplasm, slight margin around darkly stained nuclei	10–20 μ dia nuc.	Often large numbers are concentrated *Helix aspersa*
	Large leucocyte	Large amoebocyte Type B (14)	Esp. mantle tissue	Abundant cytoplasm—otherwise similar to above	10–50 μ	Actually thought to be two types, one from CT, other from mantle epithelia. *Helix aspersa*
Scaphopoda (13)	Leucocyte	Spherical leucocyte (13)	Hemolymph	Large nucleus surrounded by a strongly basophilic cytoplasm	12 μ dia	
	Leucocyte	Leucocyte (13)	Hemolymph	Spherical or kidney-shaped nuc. Weakly basophilic cytoplasm	8 μ dia	
	Leucocyte	Amoebocyte (13)	Hemolymph	Feebly basophilic cytoplasm	10–15 μ dia	
Annelida (1, 15, 16)	Basophil	Basophilic amoebocyte (15)	Coelomic fluid and sections	Vary greatly in size and shape. Rounded or oval with or w/o pseudopodia. Many with coarse basophilic granules. Small cells strain most deeply. Nuc. small, rounded often eccentric	10–5 μ dia	*Lumbricus*. All stages from lymphocyte-like to large hyaline forms. Note: large basophilic forms with acidophilic granules seen.
	Acidophil	Small acidophilic cell (15)	Coelomic fluid and sections	Usu. oval or round with pseudopodia. Nuc. well defined, central or eccentric with or w/o granules, which are usu. small	10–15 μ dia	Cell is unstable in certain media and granules may fuse
	Acidophil	Medium acidophilic cell (15)	Coelomic fluid and sections	Vary greatly in shape. Eccentric nuc. Fine and/or coarse granules. Latter may be basophilic	15–25 μ dia	
	Acidophil	Large acidophilic cell (15)	Coelomic fluid and sections	Oval to fibroblast-like shape. Fine granules usu. present often numerous. Basophilic granules or brown pigment may be seen	up to 50 μ dia	

TABLE I—(*Continued*)

Phylum and Class	General cell type	Published names and synonyms	Where observed	Morphology	Size	Properties and special characteristics
	?	Chloragogen cell (15)	Esp. in sections around gut	Packed with large rounded granules with an affinity for basophilic dyes. Nuc. small. Nucleolus present	50 μ dia	Granules appear to be lipoproteins. Cells are often phagocytized by leucocytes
	Leucocyte	Lymphocyte (16)	Coelomic fluid and sections	Cytology and stain response. resembles those of fish	4–5 μ	*Pheretima indica*. Described as a hemocytoblast. Phagocytic
	Leucocyte	Monocyte (16)	Coelomic fluid and sections	Cytology and stain response. resembles those of fish	7–10 μ	Phagocytic
	Granulocyte	Granulocyte (16)	Coelomic fluid and sections	Most numerous eosinophilic form	7–12 μ	
	Leucocyte	Lamprocyte (16)	Coelomic fluid and sections	Large leucocytes containing yellowish globular vacuoles	to 22 μ	Probably chloragogen cells
	Leucocyte	Linocyte (16)	Coelomic fluid and sections	Cytoplasm filled with single or multiple vacuoles	to 22 μ	
Sipunculida (17)	?	Urn cell	Coelomic fluid	Two separate cells. Anterior most is a vesicular cell, the base and neck of which fit into a saucer-shaped posterior ciliated glandular cell		Selectively scavenge foreign or morbid matter from the coelomic fluid
Arthropoda Insecta (18, 19, 20, 21, 22, 23, 33)	Leucoblast	Prohemocyte (19, 33)	Hemolymph	Small, pale grey, often intensely basophilic. Smallest of hemocytes	15 μ	Stem cells for plasmatocytes, granular hemocytes, cystocytes, spherule cells and adiphohemocytes (33)
	Leucocyte	Plasmatocyte (19)	Hemolymph	Basophilic and pleomorphic. Many tend to send out thin ruffled cytoplasmic extensions	8 μ dia to 6×20 μ	Develop into vermiform cells (20). Important in CT formation (22)? Usu. most abundant cell type
	Granulocyte	Granular hemocyte (19)	Hemolymph	Have many distinct usu. round, colorless or acidophilic granules. Nuc. usu. centrally located	10–20 μ	In some species, turn into intense hyaline forms *in vitro* resembling cystocytes
	Granulocyte	Cystocyte Coagulocyte (19)	Hemolymph	Very unstable and rapidly turn into bright hyaline forms and disintegrate with a small, round cartwheel-like nucleus in a weakly basophilic cytoplasm		Prob. a specialized type of granular hemocyte. Induce coagulation
	Granulocyte	Spherule cells (19) Coarsely granular cells	Hemolymph	Round to oval with many large, usu. round, non-refrigent, acidophilic, colorless or pale yellow inclusions	5–10 μ	As above. Break down to form intense hyaline forms. Important in trephocytosis, immunity (19)
	Granulocyte	Adipohemocyte (19) Spheroidocyte Lamprocyte (18, 20)	Hemolymph	Very large, refringent fat droplets and several other smaller, non-lipid granular inclusions present. Nucleus usu. eccentric	8–10 μ	Common in Lepidoptera. Similar to, but usu. smaller than body fat cells. Imp. in trephocytosis cells. (19). Prob. differentiated from plasmatocytes (18)
	Granulocyte	Crystal cell (23)	Hemolymph	Equivalent to the oenocytoids in morphological description		In *Drosophilia*

TABLE I—(*Continued*)

Phylum and Class	General cell type	Published names and synonyms	Where observed	Morphology	Size	Properties and special characteristics
	Leucocyte or granulocyte	Oenocytoid (18, 19) Large granular cell	Hemolymph	Usu. basophilic with either elaborate canaliculi or folded finely granular strands; or with distinct plate-like or rod-like crystals. 1 to 2 nuclei		Differentiated in live preps. Imp. in processes concerned in growth and molting (18), and trephocytosis (19)
	Leucocyte ?	Podocyte (19)	Hemolymph	Very large, flattened and plasmatocyte-like. 3–8+ long rigid cytoplasmic extensions	30 μ+ in each arm	Extended plasmatocytes often confused for podocytes (19), or transform into podocytes (21). Very rare (in 2 species)
	Leucocyte	Lamellocyte (23)	Hemolymph	A varient of the plasmatocyte with greater ability to encapsulate and greater cohesiveness		In *Drosophilia*
Crustacea (1, 5, 24, 25, 26, 27)	Hyaline leucocyte	Hyaline cells (25) Explosive corpuscle	Hemolymph	Nuc. deeply basophilic, central, 5–7 μ dia. Cyto. scant, light blue usu. w/o granules. Granules when present, very fine, light blue and in a perinuclear position	7–10 μ dia	Immature granulocyte (25). Many of living cells exhibit pseudopodia
						Crayfish
	Small granulocyte (neutrophil)	Small granulocyte (25)	Hemolymph	Nucleus central to eccentric. Granules vary from amphophilic to eosinophilic	9–18 μ dia	From Crayfish
	Large granulocyte (neutrophil)	Large granulocyte (25)	Hemolymph	Nucleus eccentric, 7–9 μ dia. Cyto. light-blue to colorless, densely packed with amphophilic to eosinophilic granules	18–35 μ dia	Most mature form (25). From Crayfish
	Hyaline leucocyte	Lymphoid cell (26) Hyaline (25)	Hemolymph	Round or spindle shaped. Cytoplasm slight, may contain a few granules. Round or indented nucleoate nucleus	5–10 μ	*Callinectes sapidus*. Similar to hemocytoblast (26). Some = to monocyte
		Semi-hyaline thigmotatic amoebocytic (26) Hyaline cell (25)		Shape as above. With few to many fine granules (mitochondria ?). Cytoplasm stains light blue	5–10 μ	Thigmatotic
	Acidophil Large granulocyte	Cells with coarse granules (26)	Hemolymph	Round to elongate, sometimes flattened shape. Granules vary between species, are round to spindle shaped up to 1.5 μ in dia	15–30 μ	Highly amoeboid
	Small granulocyte	Cells with medium granules (26)	Hemolymph	Granules usu. $\frac{1}{3}$ to $\frac{1}{2}$ dia of coarse type. Nuc., round to indented, usu. anucleolate. Shape variable	10–15 μ	In some species, the cells seem to disintegrate very rapidly: a possible clotting factor

TABLE I—(*Continued*)

Phylum and Class	General cell type	Published names and synonyms	Where observed	Morphology	Size	Properties and special characteristics
	Hyaline leucocyte	Explosive corpuscle (24, 26, 27)	Hemolymph	=hyaline cells, cells with medium granules		Very labile cells (27)
Echinodermata Holothuroidea (1, 28, 30, 31)	Hyaline leucocyte	Lymphocyte (29)	Coelomic fluid	Large nucleus with fine chromatin surrounded by a thin margin of hyaline cyto. Nucleoli present. Larger cells usu. with cytoplasmic inclusions. Shape, spherical to oval	4–6 μ dia	Sometimes possess one to three filaform pseudopodia. Seen in all species
	Hyaline leucocyte	Phagocyte (29) Bladder amoebocyte	Coelomic fluid	Nucleus spherical to kidney-shaped. Cyto. with a clear ectoplasm and granular endoplasm	6–14 μ dia	Phagocytic and seen in all species. Possess one or more petaloid or bladder-like pseudopodia. Filaform pseudopodia formed when in contact with a flat surface
	Granulocyte	Colorless morula cell (29) Spherule cell (21)	Coelomic fluid	Spherical when at rest. Each contains a variable no. of colorless refractive spherules in a hyaline cyto. Nucleus when seen is eccentric and 2.5 μ dia	8–20 μ dia	Blunt pseudopodia are formed. Seen in all species
	Granulocyte (pigmented)	Colored morula cell (29) Spherule cell (31)	Coelomic fluid	=to above but the spherules are colored (color variable)		
	Leucocyte ?	Fusiform cell (29)	Coelomic fluid	Elongate cells tapering at either end. Have a refractile outer cytoplasm	6–12 μ long 1–3 μ dia	Seen in all species
	Erythrocyte	Hemocyte (29)	Coelomic fluid	Variety of forms and not uniform within an individual. Nuc. about 4 μ dia. Hemoglobin carried in the cytoplasm	8–11 μ	True red blood cells. Found in only a few species
	?	Crystal cells (29)	Coelomic fluid	Possess one (usu.) to 3 large rhomboidal crystals with a thin film of hyaline cyto. surrounding the crystal. A flattened nuc.	10 $\times$8 $\times$5 μ	
	?	Vibratile cells (29) Flagellated amoebocyte (31)	Coelomic fluid	Spherical cells with flagella-length 4 $\times$ dia of cell body. Cyto. with vacuoles, dark or colored granules. A small nucleus		
Echinoidea (1, 28–32, 35) (see 29, 30, and 34 for an extensive list)	Hyaline leucocyte	Phagocyte amoeboid form (32, 35)	Coelomic fluid	=Holothuroid form—lymphocyte	7–10 μ	Amoeboid movement increases with volume of cyto (32). Have "spiked" pseudopodia (28). Are phagocytic
	Hyaline leucocyte	Phagocyte Petaloid form (32)	Coelomic fluid	=Holothuroid form—phagocyte	7–10 μ	Prob. a morphological derivative of amoeboid forms (28). Phagocytic

TABLE I—(*Continued*)

Phylum and Class	General cell type	Published names and synonyms	Where observed	Morphology	Size	Properties and special characteristics
	Granulocyte	Colorless morula cells (29) White mulberry cells (28) Colorless trephocyte (32) Colorless spherule cell (34)	Coelomic fluid	=Holothuroiorm. Vary from spherical to ovoid	10–18 μ dia	Defined for *Arbacia* sp. where they account for 42% of all cellular elements in coelomic fluid (32)
	Granulocyte	Colored morula cell (29) Red mulberry cell (28) Eleocyte (29, 30) Colored or red trephocyte (32) Colored spherule cell (34)		=Holothuroid form. Green, yellow and red spherules have been seen. Spherules in red form sometimes smaller than in other forms	7×15 μ	For *Arbacia*. Red form (eleocyte) is rich in glycogen and contains echinochrome (28). CDC-Red 48% and green 10% (32)
	?	Large spherical cells (29, 30)	Coelomic fluid	Brown tinted cytoplasm filled with granules and vacuoles	9–26 μ dia	Found only in the irregular urchins so far
	Hyaline leucocyte	Explosive cell (29)	Coelomic fluid	Pale yellow, hyaline and amoeboid cytoplasm. Numerous vacuoles in cytoplasm	9–14 μ	Found in all regular echinoids. Cells do not contain hemoglobin but do disintegrate rapidly upon contact with injured tissue (29)
	?	Hyaline hemocyte (30) Pointed yellow cell ? (28)				
	?	Vibratile cell (29, 31, 32, 34) "cellules vibratiles" (28)	Coelomic fluid	=Holothuroid form		Was shown to have a blade-like flagella. May be a precursor to phagocytes (32)
	?	Fusiform cell (28, 29)	Coelomic fluid	=Holothuroid form		
Asteroidea (1, 29, 30) (see 29 and 30 for an extensive listing)	Hyaline leucocyte	Bladder filaform phagocyte (29, 30)	Coelomic fluid	=Holothuroid form. Both types appear to be phases of one cell type		Abundant in coelomic fluid
	Granulocyte	Colorless morula cell (29, 30)		=Holothuroid form		Found in small numbers
Crinoidea (1, 5, 29, 30)	Granulocyte	Colored and colorless morula cell (5, 29)	Coelomic fluid	=Holothuroid form		
	Hyaline leucocyte	Phagocyte (29)	Coelomic fluid	Large nucleus and cytoplasm which often contains granules. Short pseudopodia and active phagocytes	11 μ dia	Cannot yet be correlated with other echinoderm leucocytes
	?	Cell with rods and granules	Coelomic fluid	Elongate and usu. vermiform. Granules and rods have an affinity for saffranin. Nucleus with no fixed position	5×30 μ	

TABLE I—(*Continued*)

Phylum and Class	General cell type	Published names and synonyms	Where observed	Morphology	Size	Properties and special characteristics
Ophiuroidea (1, 29, 30)	Hyaline leucocyte	Phagocyte (29)	Coelomic fluid	=Crinoid form		Common in *Gorgonocephalus*
	Hyaline leucocyte	Hemocyte (1, 29)	Ambulacral system only	Size variable, most are spherical	1.5–10.5 μ	Contain a heme pigment possibly hemoglobin
	Granulocyte	Colorless morula cell (29)	Coelomic fluid	=Holothuroid form		In *Ophiopholis aculeata*
		Vibratile cell (29)	Coelomic fluid	=Holothuroid form		In *Ophiopholis aculeata*
		Fusiform cell (29, 30)	Coelomic fluid	=Echinoid form		In *Gorgonocephalus*
Chordata Tunicata-Ascidia (1, 31)	Hyaline leucocyte	Amoeboid leucocyte (1, 31)	Coelomic fluid	Lymphocyte-like with some of the larger forms, highly phagocytic ("macrophages")		Morphological derivatives reticulated amoeboid cell, and various vacuolated cells (non-amoeboic) (1)
	Granulocyte (pigmented)	Mulberry cells (1, 31) Colored cell	Coelomic fluid	Contain granules—blue, orange, green or brown colors. Slow amoeboid movement		Green cells contain a vanadium pigment of unknown function

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Reeve) (Cahn, 1951) was collected from the grounds of the Western Oyster Co. in Burley Lagoon, a confined bay in southern Puget Sound near Purdy, Washington. The clams were held at the Northwest Marine Health Sciences Research Laboratory (Purdy, Washington) in large flats supplied with ultraviolet light-treated flowing sea water.

Animal preparation: fixation, cutting and staining

Animals were routinely fixed in cold Davidson's fixative (formalin-acetic acid-alcohol modified with sea water) and transferred to 50% and 70% ethanol. A few samples were fixed in gluteraldehyde-formalin in preparation for methacrylate embedding.

Blocks for paraffin embedding were taken from sagittal sections (anterior to posterior axis of the adductor muscles) or from mantle tissue, and carried through a graded series of alcohol, xylene and paraffin. Sections were cut on a rotary microtome at 5 to 7 μ (1 to 2 μ for plastic). The slides were then stained in Harris hematoxylin and eosin. Plastic embedded sections were stained with toluidine blue.

Fluid from the pericardial cavity was obtained before the clams were fixed, and the blood cells were observed in the unfixed state with phase-contrast optics or fixed, stained and observed with bright-field optics.

Fixed and stained preparations were obtained by allowing a sample (0.01 ml or greater) of blood to settle on a glass slide for approximately five minutes, fixing these cells for five minutes in 4% formalin in sea water and then rinsing the slide with 95% ethanol and allowing it to air dry. The prepared slides were stained directly after fixation with Giemsa stain diluted 1:20 with Sorensen's phosphate buffer at pH 7.5 and 3% absolute methanol. After staining for 20 minutes the slides were rinsed with pH 7.5 buffer and air dried.

Cell counts

Cell counts were conducted on five animals with a modified Neubauer counting chamber. The counts were made directly on living cells without dilution. Cell morphometrics were determined in living and fixed preparations using an eyepiece micrometer.

Differential analyses of leucocytes in connective tissue underlying midgut and style sac epithelia were conducted on clams held for 1 to 30 days and 30 to 50 days in the Purdy Laboratory. To maintain as much uniformity in the counts as possible, the leucocytes were classified, with respect to nuclear diameter, as follows: small, 1.5 to 3 μ; medium, 3 to 6 μ; and large, 6 to 8 μ. Nuclei less than or equal to 3 μ were placed in the small class while those greater than or equal to 6 μ were classed as large. Total cell size was estimated visually by the relationship of a medium nucleus to cytoplasmic radius. A cytoplasmic radius of less than one nuclear diameter designated a small cell, equal to one nuclear diameter a medium cell, and greater than one nuclear diameter, a large cell. Cells which exhibited heterochromatic DNA were differentiated in the counts from euchromatic forms. All differential cell counts were conducted with phase-contrast optics to facilitate recognition of cell boundaries.

Results and Discussion

Distribution

The leucocytes of *T. semidecussata,* like those of vertebrates, are transient cells, found in varying numbers in all tissues, including the muscle and kidney. They

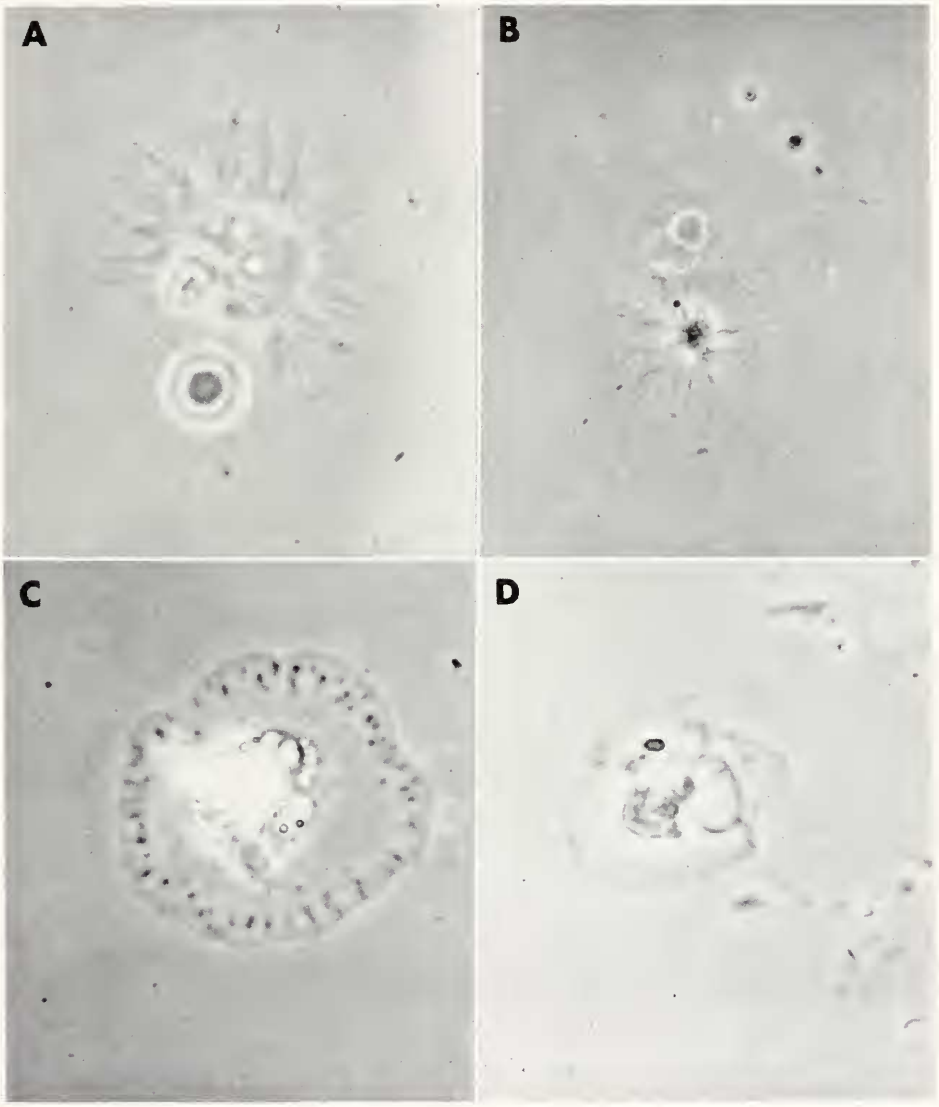

Figure 1. Living unstained leucocytes from *T. semidecussata,* A. Hyaline leucocyte with moderately filamentous pseudopodia, B. similar to A, but smaller and with greater radiation of pseudopodia, C. Hyaline leucocyte, with central granulation and peripheral cytoplasmic concentrations, D. similar to C, but without granulation and with petaloid pseudopodia, 1150 ×.

represent a distinct and identifiable group dispersed among the fibrous reticulum of loose connective tissue and between epithelial cells and muscle fibers, and are especially abundant in the subepithelial areas surrounding the GI tract, between gill lamellae and beneath the mantle epithelium. Normal appearing animals usually presented a pattern of diffusely dispersed leucocytes with some localized concentrations mainly beneath the mantle epithelium (small and medium forms) and gill lamellae connective tissue (large pigment cell forms). Hyaline and pigmented leucocytes were often infiltrated into the gastric epithelium, especially in the hindgut.

Yonge (1923) observed a similar distribution of leucocytes in the epithelium and connective tissues of the clam, *Mya arenaria*. In this regard, Haughton (1934), Wagge (1955) and Owen (1966) have reviewed literature referring to distributional patterns of leucocytes in other molluscs, especially gastropods.

Morphometrics

Many morphologically different leucocytes forms were observed both in tissue section and in fixed and living cells drawn from the pericardial cavity; however, most differences seemed to be correlated with the various preparative techniques employed. Paraffin-embedded material stained with hematoxylin and eosin, with observations mainly centered on connective tissue of the gill, mantle and GI tract, did not usually resolve the acidophilic granularity. Nuclear size, shape and position, and total cell size and shape remained as the principal gross features discriminating the cells.

The acidophilic cells in fixed suspensions generally had ovoid and eccentric nuclei, 1.4 by 2.9 microns, and the granules, when seen, were numerous. Preparations from some individuals revealed many heterochromatic nuclei in such cells. Nuclei of the hyaline leucocytes were usually round and central and varied greatly in size from 3 to 8 microns. Total cell size of these forms was extremely variable and ranged from 4 to 12 microns. At times, examples of the "pigment or brown" cells were observed in living cells, and some cells which approached the form of leucoblasts were seen. All cells seen in pericardial fluid formed pseudopodia (Figs. 1 and 2). Dissociated epithelial cells also exhibited pseudopodia formation and amoeboid movement. Granulocytic leucocytes may be distinguished in life by "spike" pseudopodia and very little motion. Hyaline leucocytes usually exhibit lobate pseudopodia and rapid motion. Both seem to be able to form branched filamentous pseudopodia (Figs. 1a, 2b).

Morphological features of the cell groups

The largest leucocytic cell observed in tissue section appeared similar to the vertebrate hemocytoblast (Bloom and Fawcett, 1962). It was infrequently seen in the Giemsa preparations and formed a homogeneous group in the maximum size range (6 to 8 μ). The next major size range, cells with medium-size nuclei (3 to 6 μ), was a heterogeneous population and represented the largest proportion of all leucocyte types found in tissue sections and in pericardial fluid. The smaller forms were the most abundant in tissue sections, but it must be noted that packing in sections does not always make them comparable with those seen in the pericardial fluid. Generally it may be concluded that the majority of medium leucocytes have

a hyaline cytoplasm in which acidophilic granules can be resolved with oil immersion in 1½ μ plastic sections. Smaller cells appear to have fewer acidophilic granules, while the largest forms are more commonly amphoteric and basophilic. The nuclei of the medium-sized cells grade rather imperceptibly into the small nuclei groups (1.5–3 μ). Between medium-sized and small cells many transitional types

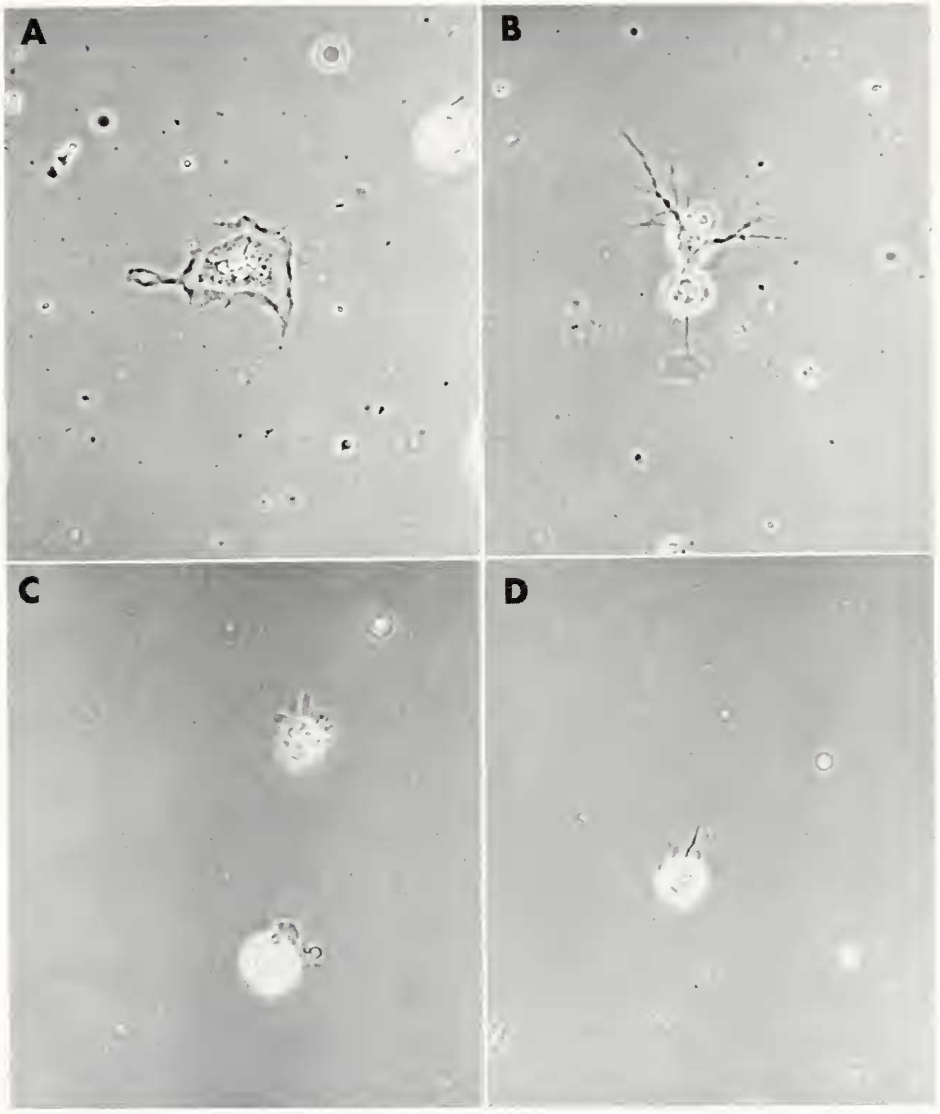

FIGURE 2. Living, unstained leucocytes from *T. semidecussata,* A. Hyaline leucocyte with lobate pseudopodia and an eccentric nucleus, B. Granulocyte with extensive filamentous pseudopodia, C. Granulocyte (lower) with hyaline cytoplasm occupying one pole, D. granulocyte with thin pseudopodia where adhered to glass; probably a morphological variant of C, 440 ×.

occur in contrast to the sharp differences noted between large and medium types. The cells with small nuclei are round or extremely elongate. Small elongate, irregular nuclei were usually euchromatic; however, heterochromatic or pycnotic round nuclei (usually 1.5–3 μ) were seen in varying proportions depending on the sample time and staining intensity. This heterochromasia was usually but not always associated with large cells (small nuclei) containing many granules. In bright field, with unstained preparations, this granularity presented a yellow to yellowish-brown coloration, the latter defined as a pigment cell character. The nuclei of all cells seemed to become more eccentric as granularity increased and the shape remained round except when compressed against the cell membrane. Except for some large leucocytes, nucleoli were not visible.

TABLE II

Differential counts of connective tissue leucocytes in the Manila clam, Tapes semidecussata. *Group I (n = 14) was held for 1 to 30 days in running seawater. Group II (n = 14) was held for 30 to 50 days in the same system. 100 cells were counted per individual*

Group	Nucleus dia (μ)	Cell dia (μ)	Mean %	S.D. ±%	Subgroup total %	Group	Nucleus dia (μ)	Cell dia (μ)	Mean %	S.D. ±%	Subgroup total %
I		3–6	11.3	5.9		II		3–6	14.0	7.1	
	1.5–3	6–12	19.5	5.5	40.2		1.5–3	6–12	32.1	12.8	59.0
		12–16	9.4	3.5				12–16	12.9	4.7	
		3–6	31.6	12.3				3–6	21.1	9.8	
	3–6	6–12	18.4	6.2	53.9		3–6	6–12	12.9	7.3	37.8
		12–16	3.9	2.2				12–16	3.8	2.9	
		3–6	1.2	1.5				3–6	1.0	1.3	
	6–8	6–12	2.9	1.7	5.9		6–8	6–12	1.5	1.9	3.2
		12–16	1.8	1.3				12–16	0.7	1.3	
	1.5–3*	All	24.5	6.6			1.5–3*	All	40.0	13.2	

* Heterochromatic nuclei.

Changes in the proportions of leucocytes possibly due to prolonged storage of clams under artificial conditions are shown in Table II. A comparison of differences in relative numbers between cell sizes and groups held for 1 to 30 days and 30 to 50 days suggest an increase in small-heterochromatic forms in the latter group. Tests employing the single classification analysis of variance were conducted to determine the significance of differences between groups for each nuclear size classification shown in Table II. The significance levels for acceptance of the hypothesis that the means of both groups were similar ($M_1 = M_2$) were as follows: all small forms, $P < 0.001$ ($F = 18.74$); heterochromatic small forms, $P < 0.001$ ($F = 19.68$); medium forms, $P < 0.005$ ($F = 10.57$); and large forms, not significant ($F = 2.31$). The heterochromasia exhibited by many small cells probably indicates some loss of cell function or degeneration (Robbins, 1967). The gross and histologic appearance of both groups was similar.

Summary

The morphology and known functions of leucocytes have been tabulated and compared for most invertebrate phyla. The leucocytes of the Manila clam are similar in form to molluscan leucocytes described by other investigators. Two general types were seen. One was a hyaline leucocyte ("leucocyte") and the other a granular leucocyte ("granulocyte"). Cells in these categories showed extreme variations in size and staining characteristics. A decrease in nuclear size often was correlated with an increase in nuclear heterochromasia and eccentricity and cytoplasmic granularity. The average cell count for all circulating leucocytes was 1650 ± 180 per mm^3.

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